International Journal of Bio-Technology and Research (IJBTR)
ISSN(P):2249-6858; ISSN(E):2249-796X
Vol. 7, Issue 1, Feb 2017, 11-18

TRANS STELLAR
•Journal Publications •Research Consultancy

ESTABLISHMENT OF MULTIPLICATION PROTOCOL FOR INSULIN PLANT *(COSTUS PICTUS D. DON) BY* USING STEM NODE AS EXPLANTS

AFZAL KHAN[1], VIJAY SHARMA[2] & R. K. SHUKLA[3]

[1]*Assistant Manager (QC and R&D), Patanjali Bio Research Institute, Village, Padartha, Haridwar, Uttarakhand, India*

[2]*Head of Department (QC and R&D), Patanjali Bio Research Institute, Village, Padartha, Haridwar, Uttarakhand, India*

[3]*Head of Department (Production), Patanjali Bio Research Institute, Village, Padartha, Haridwar, Uttarakhand, India*

ABSTRACT

Costus pictus D. Don is a member of family Costaceae and commonly known as Insulin plant. A lot of research has been conducted to evaluate the anti-diabetic potential of this plant and revealed that a bioactive molecule tetracosonoate extract from Costus pictus leaves contain anti-diabetic activity. It is vulnerable species in India and due to indiscriminate collection now on the verge to extinction. It is found that the axillary buds of Costus pictus are dormant which limits it asexual propagation. Tissue culture may promise to preserve, protect and multiply this rare species for large scale multiplication. In the present study, a simple protocol for rapid mass propagation of Costus pictus was standardized using various concentrations of plant growth regulators like naphthalene acetic acid (NAA), 6-benzylaminopurine (BAP) and urea with Murashige and Skoog (MS) medium. The dormancy of axillary buds were successfully broken in stems cultured in MS medium with 1 ppm BAP, 0.5 ppm NAA (79.0%) and MS medium with 2 ppm BAP (66%). These sprouted in vitro axillary buds were used as secondary explants for shoot multiplication. The significantly highest numbers of shoots (6.67±0.65) per explants, shoot length (3.62±0.26 cm), roots number (9.71±0.52) per plantlet and root length (5.16±0.22 cm) was observed on MS medium supplemented with 1 ppm NAA, 4 ppm BAP and 10 ppm Urea. Primary and secondary hardening was done successfully. The micro-propagated plantlets do not show any visible morphological abnormalities.

KEYWORDS: Costus Pictus, Anti-Diabetic, Micropropagation, Nodal Segment & Bud Induction

Received: Dec 27, 2016; **Accepted:** Jan 21, 2017; **Published:** Jan 27, 2017; **Paper Id.:** IJBTRFEB20172

Original Article

INTRODUCTION

Costus pictus D. Don (syn. *Costus igneus* Nak, *Costus mexicanus* or *Costus congenitus*), a perennial herbs belonging to Costaceae (Zingiberaceae) family, grows up to height of two to three feet[1].Popularly known as Insulin plant where as its botanical name is *Costus pictus D. Don*[1-2]. The flowers appear on branches tip and orange in colour, ultimately develops into cone like fruits[3]. It originally belong South and Central America (Mexico) and was introduced to India[4] . In southern India, it usually grows as an ornamental plant and its leaves are used as a dietary supplement in the treatment of diabetes mellitus[5-6]. The aerial part of Costus *pictus* is used as an infusion for the treatment of renal disorder by the practitioners in Mexico[7]. The leaf of this plant was reported to contain many antioxidant components likes ascorbic acid, flavonoids and also rich source of iron[8]. A study conducted by Nandhkumar J et al.,2007 on rat, and a similar study conducted by Remya R and Daniel M, 2012, revealed that the methanol/water extract of this plant contain many phytochemicals such as Carbohydrates, Proteins, Alkaloids, Tannins, Glycosides, Saponins, and Flavonoids[9-10]. A lot of research work has been conducted on time to time and proven that majority of the chemical extracted from *Costus pictus* and its related species are

anti-diabetic in nature[7, 9, 11-21]. Also it has been proven by many studies that the extract of *C. pictus* has therapeutic potential such as Anti-cancer, Hypolipidemic, Anti-microbial, Diuretic, Ameliorative, activity[4, 15, 22-25]. *Costus pictus* D. Don is an important rare medicinal plant and about to extinction due to its habitat destruction, deforestation, over exploitation and indiscriminate collection for commercial uses. Its conventional propagation is very deliberate because the seeds are nonviable, low rate of seed germination and low successes rate of vegetative propagation[2]. Alternative methods like Plant Tissue Culture will be helpful to achieve large rate of multiplication which leads to its conservation. Very limited study for the micropropagation has been done and multiplication rate was not very satisfactory. The present experiment was conducted to establish a simple and efficient protocol for micropropagation of *Costus pictus* D. Don and emphasized to achieve higher rate of multiplication.

MATERIALS AND METHODS

The nodal segment and rhizome segment used as explants in this study were collected from the experimental field of Patanjali Bio Research Institute, Haridwar, Uttarakhand, India. These explants were first kept under running tap water for 10minutes and then washed in double distilled water with 2-3 drops of Tween 20 for 10 minutes. These explants were further sterilized with0.1% (w/v) mercuric chloridefor 3-4 minutes under the laminar flow after which they were rinsed thrice with sterile distilled water. Explants were given a fresh cut and made into pieces ranging in size from 1.0 to 1.5 cm long and inoculated on to MS medium[26] supplemented with various Plant Growth Regulators (PGRs), sucrose 30 g L-1 and 8 g L-1 agar. The pH of the media was adjusted to 5.8 before being autoclaved at 121°C for 20 min and 15 lbssq inch-1 pressures. All cultures were incubated at 25±2°C under 24 hr (day/night) photoperiod for first 40 days and then 10 hr for rest of period with light supplied by white fluorescent tubes (2500 lux). Subculturing was carried out after every 5 weeks by trimming-off roots.

Culture Establishment and Bud Initiation

MS media supplemented with 1 ppm Benzyl Amino Purine (BAP), 0.5 ppm Naphthalene Acetic Acid (NAA) in combination with 3% sucrose and MS medium supplemented with 2 ppm BAP in combination with 3% sucrose were tested in case of *Costus pictus* D. Don. Fifteen replicates were taken for the above experiments and repeated twice.

Shoot Multiplication and Rooting

After 2 weeks on initiation medium, the initiated buds were excised and trimmed upto 0.4-0.6 cm long before to transferred in MS medium supplemented with 3% sucrose in combination with 2 ppm BAP. The shoot buds obtained from above medium of *Costus pictus* D. Don were excised after 2 week and subcultured in medium supplemented with 4 ppm BAP. Finally the contaminated explants were eliminated and only healthy shoot buds were taken to subcultured in MS medium supplemented with 3% sucrose with three different PGRs combination viz. 1 ppm NAA + 4 ppm BAP + 10 ppm Urea, 2 ppm BAP and 4 ppm BAP. The shoot buds were subcultured twice at the interval of 40 days on the same medium combination. Ten replicates were taken for the above experiments and repeated twice.

Statistical Analysis

The statistical analysis was accomplished by using the SPSS statistical software package and the data were recorded after 10 weeks of bud initiation. Data were examined for any significance using ANOVA and the differences contrasted using Tukey's comparison tests at 1% and 5% probability test.

RESULTS

Initiation of bud

Rhizome (basal) nodes and stem nodes were collected during the month of October, 2014. Explants collected during April-September showed bud initiation within 20-25 days but those collected during October showed bud initiation after 10-15 days. The basal part showed bud induction frequency significantly higher than stems cultured on the same day of collection, however, fungal and bacterial contamination was higher in basal part than stems. Single nodal explants were cultured on MS media supplemented with 1 ppm BAP, 0.5 ppm NAA in combination with 3% sucrose and MS medium supplemented with 2 ppm BAP in combination with 3% sucrose. The bud induction frequency (79%) was achieved for the combination of 0.5 ppm NAA and1 ppm BAP in comparison to 66 % for 2 ppm BAP supplemented MS medium. (Table 1, Figure 1a).

Effect of Growth Regulators Combination on Shoot Multiplication from Single Explants

After 25 days on bud initiation, the developed auxiliary buds were transferred on MS medium supplemented with different combination of growth regulators (BAP, NAA and Urea) for further growth and proliferation. The excised buds were trimmed before to transfer on fresh medium. A clear cur shoots differentiation were observed in transferred buds within two week in all PGRs combination. Also swelling was found at the base of transferred bud within 7-10 days. The buds were cultured on the same media for another 10 weeks and data were recorded for multiple shoots. The best shoot multiplication response per explants was accomplished in 1 ppm NAA, 4 ppm BAP and 10 ppm Urea (Table 2). In this medium 6.67±0.65 shoots (average) were obtained from a single bud whereas 3.05±0.17 shoots were recorded for medium supplemented by 4 ppm BAP. Minimum average number of shoots (2.1±0.20) was observed for medium supplemented with 2 ppm BAP.

Effect of Growth Regulators Combination on Shoot Length

The different combination of growth regulators in present or absent of Urea had significant effect on the shoot length. Proliferated multiple shoots of about 0.5 -1.0 cm long were excised and sub-cultured on agar gelled MS medium containing different combination of growth regulators viz. 2 ppm BAP, 4 ppm BAP and 1ppm NAA, 4 ppm BAP with 10 ppm Urea. The longest shoots (3.62±0.26 cm long) was observed in medium supplemented with 1 ppm NAA, 4 ppm BAP and 10 ppm Urea followed by 2.87±0.22 cm for medium supplemented by 4 ppm BAP (Table 2). Minimum average length of shoot (1.67±0.18 cm) was recorded for medium supplemented with 2 ppm BAP

Effect of Growth Regulators Combination on Roots Number and Length

Shoots on the medium supplemented with 1 ppm NAA, 4 ppm BAP and 10 ppm Urea were continued for next 4 weeks and root number were scored. The regenerated multiple shoots (1.4-2.9 cm long) on medium supplemented 2 ppm BAP and 4 ppm BAP were excised and transferred to MS medium fortified with 1 ppm NAA. After 2 weeks roots induction was observed and found that the developed roots were small and hairy. After 4 weeks of root development the root number and length were recorded. The maximum average number of roots (9.71±0.52) was observed in shoot continue cultured on medium supplemented with 1 ppm NAA, 4 ppm BAP and 10 ppm Urea in comparison to the shoots transferred from 2 ppm (4.80±0.20) and 4 ppm (4.80±0.30) BAP to 1 ppm NAA (Table 2, Figure 1c). Also the plantlets on 1 ppm NAA, 4 ppm BAP and 10 ppm Urea supplemented media had longer roots than those transferred from 2 ppm and 4 ppm BAP to 1 ppm NAA (Table 2). The maximum average length of roots in 1 ppm NAA + 4 ppm BAP + 10 ppm Urea

supplemented medium was 5.16±0.22 cm, whereas it was 1.84±0.13 cm forshoot transferred from 2 ppm BAP to 1 ppm NAA supplemented medium and 2.5±0.17 cm for shoot transferred from 4 ppm BAP to 1 ppm NAA supplemented medium (Table 2). The maximum average number of multiple shoots was observed in regenerated shoot continued to culture on medium which had already supplemented with 1 ppm auxin in comparison to shoots which were transferred from the cytokinin medium to auxin medium in the same duration.

Hardening of Plantlets

The rooted plantlets were taken out from the culture and washed with water carefully to remove the traces of agar and they were transplanted in culture (jam) bottles containing autoclaved mixture of Co-copit, Perlite and Vermicompost (4:2:1) for primary hardening and incubated in the same culture conditions. There was 90% survival of plants in this condition. The well grown plants were then transferred to plastic pots containing sterile mixture of Sand, Soil and Vermicompost (1:3:2) for secondary hardening (Figure 1e). The plantlets were irrigated with tap water twice in a week for a total period of two weeks to complete the acclimatization process. There was 80% survival of plants during secondary hardening.

CONCLUSIONS

Nodal segment culture has been successfully reported in numbers of medicinal and ornamental plants like *Stevia rebaudiana*[27-28], *Withania somnifera*[29-30], *Coleus forkohlii*[31] etc and tree species like *Wrightia tomentosa*[32], *Azadiracta indica*[33] and also in bamboo species like *Dendrocalamus longispathus*[34] and *D. giganteus*[35]. It has already been reported that the nodal segments of *Costus speciosus* show bud initiation and shoot multiplication in medium supplemented with BAP, NAA and AdS[36]. The present study reveals that, both bud initiation and shoot multiplication is possible in media supplemented with BAP, NAA and Urea. During this experiment, it was observed that bud initiation frequency was higher in stem which had flowers and was fully mature. The higher frequency of bud break in the nodal segment from lower portion of the stem was reported. In conclusion, the study conducted offers a potential system for conservation and mass propagation of *Costus pictus* D. Don from pre-existing nodal meristems as explants. MS medium containing 1 ppm NAA + 4 ppm BAP+ 10 ppm Urea is the best for shoot proliferation. MS basal medium supplemented with 1ppm IAA is the best for root induction. The use of nodal segment provides an alternative source for micropropagation of *Costus pictus* and can be beneficial than other explants (Rhizomes, leaves etc.).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support received from Acharya Balkrishna, Vice Chancellor University of Patanjali, Dr. Yashdev Shastri, Patanjali Gram Udyog Nyas, Mr. Ram Bharat & Swami Muktanand, Patanjali Ayurved Ltd., for carrying out this research work and the various staff of Patanjali Bio Research Institute, Gram Udyog Nyas, Patanjali Ayurved Ltd. and Patanjali Food & Herbal Park for assistance during different stages of research studies

REFERENCES

1. *Specht DC, Stevenson WD, A new phylogeny-based generic classification of Costaceae (Zingiberales). Taxon, 55 (1) (2006) 153.*
2. *Bakrudeen Ali AA, Arun KR, In vitro propagation of Monocot (Costus pictus D. Don) – An antidiabetic medicinal plant. J Agri Technol, 5(2) (2009) 361.*

3. *Gilman EF, Costus igneus. Fact sheet. Florida: University of Florida, Inc; c. FPS-151. EDIS-Electronic Data Information Source-UF/IFAS Extension (2012).*
4. *Beena J, Joji LR, Analysis of the essential oils of the stems, leaves and rhizomes of the medicinal plant Costus pictus from southern India. Int J Pharmacy Pharm Sci, 2(2) (2010) 100.*
5. *Merina B, Insulin plant in gardens. Natural Product Radiance, 3 (2004) 349.*
6. *Vishalakshi DD, Asna U, hypoglycemic potential of Morus indica. L and Costus igneus. Nak: A preliminary study. Indian j Exp Biol, 46(2008) 614.*
7. *María Estela MC, Rocío CN, Rafael ST, María-Elena CA , Evaluation of the diuretic effect of the aqueous extract of Costus pictus D. Don in rat. Proc West Pharmacol Soc, 49 (2006) 72.*
8. *Vishalakshi DD, Asna U, Nutrient profile and antioxidant components of Costus specious Sm. and Costus igneus Nak. Indian J Nat Pro and Reso, 1(2010)116.*
9. *Nandhkumar J, Sethumathi-Pudhupalayam P, Malini A, Sengottuvelu S, Duraisami R, Karthikeyan D, Thangavel S, Anti-diabetic activity of methanol leaf extract of Costus pictus D. Don in alloxan-induced diabetic rats. J Health Sci, 53(2007) 655.*
10. *Remya R and Daniel M, Phytochemical and pharmacognostic investigation of antidiabetic Costus pictus. D. Don. Int J Pharm and Biomed Res, 3(1) (2012)30.*
11. *Jayasri MA, Gunasekaran S, Radha A, Mathew TL, Anti-diabetic effect of Costus pictus leaves in normal and streptozotocin-induced diabetic rats. Int J Diabetes and Metabolism, 16 (2008)117.*
12. *Sethumathi PP, Nandhakumar J, Sengottuvelu S, Duraisamya R, Karthikeyan D, Ravikumar VR, Malinib A, Sivakumara T, Antidiabetic and antioxidant activity of methanolic leaf extracts of Costus pictus D. Don in alloxan induced diabetic rats. Pharmacologyonline, 1(2009)1200.*
13. *Vishnu B, Naveen A, Akshay K, Mukesh-Sikarwar S, Patil MB, Antidiabetic activity of insulin plant (Costus igneus) leaf extract in diabetic rats. J Pharmacy Res, 3(3): (2010) 608.*
14. *Panagal M, Arun RK, TMM John B, Jenifer S, Muthuvel A, Comparative evaluation of extracts of C. igneus (or C. pictus) for hypoglycemic and hypolipidemic activity in alloxan diabetic rats. Int J Pharmacy Tech, 2(2010)183.*
15. *Al-Romaiyan A, Jayasri MA, Mathew TL, Huang GC, Amiel S, Jones PM, Persaud SJ, Costus pictus extracts stimulate insulin secretion from mouse and human islets of Langerhans in vitro. Cell Physiol Biochem, 26 (2010)1051.*
16. *Akhila JS, Divya C, Rejeesh VN, Nair V, Kuruvilla M, Shashidhar K, Effect of the insulin plant (Costus igneus) leaves on dexamethasone-induced hyperglycemia. Int J Ayurveda Res, 1 (2) (2010) 100.*
17. *Stabitha TI, Alphonse JK, JK Mini A, Comparative study of hypoglycemic activity of Costus pictus and Costus igneus in streptozotocin induced diabetic rat. J Pharm Res, 4(2011) 3628.*
18. *Pazhanichamy K, Aishwarya DS, Jeba-Samuel CS, Priya G, Yogha G, Manjula K, Rajendran K, The efficacy of Costus igneus rhizome on carbohydrate metabolic, hepatoproductive and antioxidative enzymes in steptozotocin-induced diabetic rats. J Health Sci, 57(2011)37.*
19. *Pazhanichamy K, Rajendran K, Kumaran S, Efficacy of methanolic extract of Costus igneus rhizome on hypoglycemic, hypolipidimic activity in streptozotocin diabetic rats and HPTLC analysis of its active constituents. International Conference on Bioscience, Biochemistry and Bioinformatics, 5(2011) 318.*

20. *Vishalakshi DD, Asna U, Evaluation of anti-hyperglycemic and anti-lipid peroxidative effect of Costus igneus Nak in streptozotocin induced diabetic rats. Int J Curr Res, 33(2011)4.*
21. *Suganya S, Narmadha R, Gopalakrishnan VK, Devaki K, Hypoglycemic effect of Costus pictus D. Don on alloxan induced type 2 diabetes mellitus in albino rats. Asian Pacific J Tropical Dise, 2(2012)117.*
22. *Nimmy C, Shastry CS, Prerana S, Prasanna S, Ullas D, Patel M, Anti hyperlipidemic activity of Costus igneus in Triton X-100 induced hyperlipidemic rats. Int J Pharm and Chem Sci, 1(2012)813.*
23. *Mala M, Prachi SP, Antibacterial, antioxidant and antiglycation potential of Costus pictus from southern region of India. Asian J Plant Sci Res, 2 (2012) 95.*
24. *Malairaj S , Anadhan V, Ramar T, Perumal P, Ramasamy R, Kandasamy M, In vitro antioxidant and anticancer potential of bark of Costus pictus D. Don. Asian Pacific J Tropical Biomedicine, 2 (2012)741.*
25. *Manjula K, Pazhanichamy K, Shanmugam K, Tamilmani E, Dale KC, Rajendran K, Growth characterization of calcium oxalate monohydrate crystals influenced by Costus igneus aqueous stem extract. Int J Pharm Pharm Sci, 1 (2012) 261.*
26. *Murashige T, Skoog FA, revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant, 15 (1962) 473.*
27. *Yau-Wen Y, Yue-Ie H, Wei-Chin C, Clonal propagation of Stevia rebaudiana Bertoni through axillary shoot proliferation in vitro. Bot. Bull. Academia Sinica, 22(1981)57.*
28. *Pande SS, Priyanka G, Plant tissue culture of Stevia rebaudiana (Bertoni): A review. J Pharmacognosy and Phytotherapy, 5(1) (2013) 26.*
29. *Narendra C, PC Trivedi, In vitro Regeneration of Plantlets via Shoot tip Culture in Withania somnifera L. Elixir Appl Botany, 45 (2012)7850.*
30. *Sharmistha G, Pankaj K. Sahub, In vitro micro propagation of Withania somnifera (L.) Dunal. Saudi J Biolo Sci, (2015) (in press).*
31. *Pratibhadube, Moumita G, Saikat D and M-Nasim A, Establishment of rapid multiplication protocol of Coleus forskihlii Briq. And invitro conservation by reduced growth. Indian J Biotec, 10 (2011) 228.*
32. *Sunil DP, Gotam K, Sharma P, Kiran T, In vitro propagation of an adult tree Wrightia tomentosa through enhanced axillary branching. Plant Sci, 103(1994)67.*
33. *Sarita A, Trilok RS, Inder DA, In vitro propagation of neem from seedling and mature tree. Indian J Plant Gene Resou, 8(2) (1995)247.*
34. *Sanjay S, Sant SB, In vitro clonal multiplication of 4 year-old plants of the bamboo, Dendrocalamus longispathus Kurz. In vitro Cell Devl Biol Plant, 29(1993)134.*
35. *Ramanayake SMSD, Kapila Y, Micropropagation of the giant bamboo (Dandrocalamu sgigangteus Munro.) from nodal segments taken from field grown culms. Plant Sci, 129(1997)213.*
36. *Kshetrimayum P, Jitendra GS, Micropropagation of Costus speciosus (Koen) Sm. using nodal segment culture. Notulae Scientia Biologicae , 2(1) (2009) 58.*

APPENDICES

Table 1: Effect of Different Levels of BAP Alone or in Combination with NAA on Bud Induction of Costus Pictus

Treatment (PPM)			Bud Break (%)
BAP	NAA	Sucrose (%)	
1	0.5	3	79.0
2		3	66.0

Table 2: Effect of Different Levels of BAP Alone or in Combination with NAA and Urea on Shoot Multiplication and Rooting of Costus Pictus in MS Medium. Means Followed by Same Letters in Column are Not Significantly Different at P<0.05, According to Tukey's HSD Comparison Test

Treatment (ppm)			No. of Shoots (Mean ± S.E.)	Shoot Length (cm) (Mean ± S.E.)	No. of Roots (Mean ± S.E.)	Root Length (cm) (Mean ± S.E.)
BAP	NAA	Urea				
4#	**1**	**10**	6.67±0.65a	3.62±0.26a	9.71±0.52a	5.16±0.22a
4**			3.05±0.17b	2.87±0.22b	4.80±0.30b	2.5±0.17b
2*			2.1±0.20c	1.67±0.18c	4.80±0.20cb	1.84±0.13c

Culture continued on same media without subculture.

Transfer from 2 ppm of BAP to 1 ppm NAA for root induction

Transfer from 4 ppm BAP to 1 ppm NAA for root induction

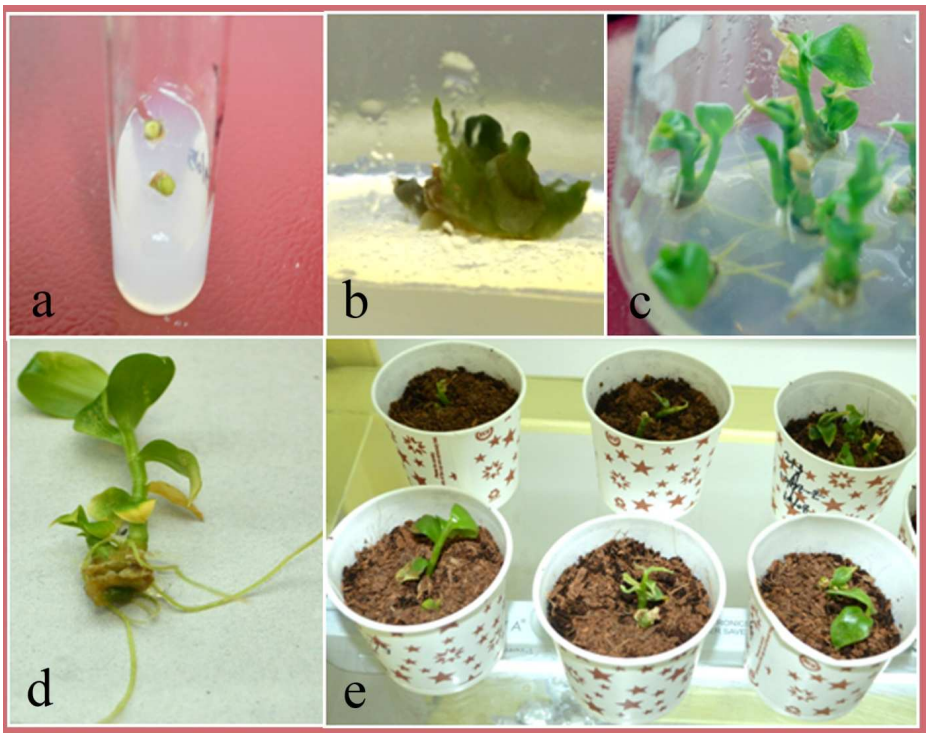

Figure 1: Micropropagation of *Costus Pictus* in MS Medium with 4 Ppm BAP + 1 Ppm NAA + 10 Ppm Urea (A) Axillary Bud Sprouting from Node Segments (B) Axillary Shoot Proliferation, (C) Root Induction in Plantlets, (D) Single Plantlets during Hardening, (E) Secondary Hardened Plantlets